

srRNA-seq analysis of human embryonic stem cells and definitive endoderm reveal differentially expressed microRNAs and novel isomiRs with distinct targets.

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Public Summary:

MicroRNAs (miRNAs) are noncoding, regulatory RNAs expressed dynamically during differentiation of human embryonic stem cells (hESC) into defined lineages. Mapping developmental expression of miRNAs during transition from pluripotency to definitive endoderm (DE) should help to elucidate the mechanisms underlying lineage specification and ultimately enhance differentiation protocols. In this report, next generation sequencing was employed to build upon our previous analysis of miRNA expression in human hESCs and DE. From millions of sequencing reads, 747 and 734 annotated miRNAs were identified in pluripotent and DE cells, respectively, including 77 differentially expressed miRNAs. Among these, four of the top five upregulated miRNAs were previously undetected in DE. Furthermore, the stem-loop for miR-302a, an important miRNA for both hESC self-renewal and endoderm specification, produced several highly expressed miRNA species (isomiRs). Overall, isomiRs represented >10% of sequencing reads in >40% of all detected stem-loop arms, suggesting that the impact of these abundant miRNA species may have been overlooked in previous studies. Because of their relative abundance, the role of differential isomiR targeting was studied using the miR-302 cluster as a model system. A miRNA mimetic for miR-302a-5p, but not miR-302a-5p(+3), decreased expression of orthodenticle homeobox 2 (OTX2). Conversely, isomiR 302a-5p(+3) selectively decreased expression of tuberous sclerosis protein 1 (TSC1), but not OTX2, indicating non-overlapping specificity of miRNA processing variants. Taken together, our characterization of miRNA expression, which includes novel miRNAs and isomiRs, helps establish a foundation for understanding the role of miRNAs in DE formation and selective targeting by isomiRs. Stem Cells 2014.

Scientific Abstract:

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